

Abnormal Chromosome Repair and Risk of Developing Cancer

by William W. Au¹

Several scientists have proposed that DNA repair deficiencies and the induction of a mutator phenotype are responsible for the generation of multiple mutagenic alterations in cancer cells. I propose that exposure to environmental carcinogens can induce DNA lesions, elicit infidelity of DNA repair, and cause the instability phenomenon and the subsequent consequences. Using cell lines derived from mammary glands of irradiated mice, my laboratory conducted sequential studies to document genetic events leading to the development of malignant cells *in vitro*. We found that aneuploidy and extensive chromosome breaks and rearrangements occurred early. This is followed by inactivation of the retinoblastoma tumor-suppressor gene, amplification of the *myc* oncogene, and expression of the tumorigenic phenotype. Our observation of chromosome instability at the early phase of transformation is consistent with the mutator phenotype. We suggest that a cause of the instability is infidelity of DNA repair, and we have developed a challenge assay to elucidate this phenomenon. In this assay, cells are challenged to repair radiation-induced DNA lesions. In one of our studies, lymphocytes from cigarette smokers and nonsmokers were exposed to γ rays *in vitro*. Cells from smokers had significantly more rearranged chromosomes than cells from nonsmokers after the challenge. These data suggest that smokers have infidelity of DNA repair and that this repair problem is a cause of health effects in smokers. In an *in vitro* study, lymphocytes were exposed to mitomycin C or to nickel acetate and then irradiated with γ rays. Significantly increased frequencies of rearranged chromosomes were detected with low doses of mitomycin C and nickel, which do not cause chromosome damage by themselves. Lymphocytes from patients with epidermodysplasia verruciformis were found to have infidelity of DNA repair after exposure to UV-light in our challenge assay. This sensitivity is consistent with the predisposition to skin cancer after exposure to sunlight. Our studies suggest that infidelity of chromosome/DNA repair can generate an unstable genome, which allows genetic alterations relevant to the development of cancer to evolve. Furthermore, infidelity of chromosome/DNA repair allows the generation of multiple genetic alterations in the multistep carcinogenic process.

Introduction

It is well documented that the majority of cancers from human have chromosome abnormalities (1,2). Furthermore, single and specific chromosome abnormalities are consistently associated with certain cancers. For example, Burkitt's lymphomas usually have a translocation between chromosomes 8 and 14. Retinoblastomas often have a deleted chromosome 13. These nonrandom cytogenetic changes clearly indicate that alteration of specific genes is the cause of certain cancers. Indeed, extensive searches using molecular techniques have concluded that development of Burkitt's lymphoma and retinoblastoma involves amplification of the *myc* oncogene and inactivation of the *Rb* tumor-suppressor gene, respectively (3,4). The cytogenetic abnormalities observed in most other cancers, however, involve more than one chromosome. Therefore, alteration of several genes may be required for development of these cancers.

The relationship between these chromosome changes and alteration of oncogenes in human cancers are summa-

rized by Yunis and Soreng (5) and LeBeau (6). It is obvious that carcinogenesis is a complex, multistep process (7). In most cases, a sequence of specific genetic alterations is required to convert a normal cell through a multistep process and clonal expansion to become a cancer cell. It is therefore necessary to have a good understanding of the carcinogenic process to precisely estimate the risk for developing cancer. In this paper, some factors that contribute to the development of cancer are briefly summarized. In addition, the use of our challenge assay to detect infidelity of DNA repair and as a biomarker for risk of developing cancer is presented.

Genetic Factors for Developing Cancer

Multicellular organisms have a host of genes that regulate cell growth and cell-cell interactions. Alteration of these genes may lead to abnormal cell differentiation and proliferation. Inheritance of these abnormal genes may, therefore, predispose an individual to an increased risk of developing cancer.

One of these genes is the retinoblastoma (*Rb*) gene. In 1971, Knudson predicted, based on statistical analyses, that two mutational events were sufficient for the develop-

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ment of retinoblastoma (8). Patients with predisposition to develop this tumor would have inherited one of the two mutational events, therefore, they would develop the tumor early in life and would have multiple tumors. The primary mutational event was documented cytogenetically by Yunis and Ramsay (9) to be a specific deletion in chromosome 13. Using molecular techniques, Friend et al. (10), Lee et al. (11), and Fung et al. (12) simultaneously identified the DNA sequence that is deleted in *Rb* patients. This sequence and its corresponding gene is known as the *Rb* gene. The primary function of this gene is to control cell proliferation (13). The two mutational events as predicted by Knudson are shown to be homozygous deletion of the *Rb* gene. Because inactivation of this gene is necessary for the development of cancer, this and other genes with similar involvement in carcinogenesis are classified as tumor-suppressor genes (14,15).

Inheritance of one defective tumor-suppressor gene would increase the chance of homozygous inactivation of the gene and would therefore significantly increase the risk of developing retinoblastoma. On the other hand, two sequential *de novo* mutations that inactivate a tumor-suppressor gene (e.g., homozygous deletion) can also occur, albeit with much reduced frequency. This is, in fact, observed in *Rb* patients who have not inherited the defective *Rb* gene from their parents. Because the probability for two sequential hits to inactivate a gene is low, these patients usually have retinoblastoma later in life than those who have already inherited one defective *Rb* gene.

Damage is frequently induced in DNA on a daily basis, but cells have excellent DNA repair enzymes that correct the damage and restore normal cellular functions. Inheritance of genes that code for defective DNA repair enzymes would cause mistakes during the DNA repair process and would predispose the individuals to develop cancer. For example, patients with xeroderma pigmentosum are unable to repair UV-induced damage in cellular DNA, and they have a high incidence of skin cancer (16). Other less well-characterized patients are those with Bloom syndrome, Fanconi anemia, and ataxia telangiectasia. These diseases are recessively inherited syndromes with a predisposition to develop cancer. Although the homozygotes for these diseases can be easily identified, the heterozygotes are phenotypically normal and are indistinguishable from normal individuals. However, Swift et al. (17-19) have demonstrated that these heterozygotes also have increased risk to develop cancer compared to the general population. Therefore, these defective genes can also be genetic risk factors in the heterozygous state. The extent of their contribution to cancer incidence among the phenotypically normal population remains to be determined.

Our environment contains natural and man-made hazardous agents, and exposure to them may cause cancer. Complex organisms have developed defense mechanisms against the assault by xenobiotics. One of the defense mechanisms is the use of metabolizing enzymes to break down reactive chemicals and consequently inactivate them. Unfortunately, some chemicals are activated in this metabolizing process. An example is the metabolic activation of polycyclic aromatic hydrocarbons by cytochrome

P-450 enzymes (20) into chemicals that can interact with DNA. These enzymes are inducible, and their inducibility varies from one individual to the next. It is therefore believed that individuals with high inducibility of these enzymes would have increased risk to develop cancer from exposure to polycyclic aromatic hydrocarbons and other chemicals that require similar enzyme systems for activation. Indeed, this relationship was demonstrated recently by Bartsch et al. (21), who reported that the inducible phenotype is associated with risk of developing lung cancer among cigarette smokers. These genetically regulated metabolic activities are therefore risk factors for developing cancer from exposure to environmental carcinogens.

Environmental Factors for Developing Cancer

Physical, chemical, and biological agents that can cause cancer are present in our environment. Some of these agents exist naturally, and others are produced by human activities. Ionizing radiation is one of the earliest documented human carcinogens. Among biological agents, viruses such as papillomavirus and hepatitis B virus are believed to be responsible for a significant portion of cancer incidence worldwide (22). On the other hand, in developed and in many developing countries, cigarette smoking is generally recognized to be the most significant cause of cancer in human. In addition to smokers, nonsmokers who are exposed to sidestream smoke from burning cigarettes may also have an increased risk of developing cancer (23).

Human carcinogens are also present in workplaces. The International Agency for Research on Cancer (24) has documented these carcinogens. Scientists are actively seeking out environmental factors that can cause cancer because removal of the identified factors are effective preventive measures against cancer development.

In our laboratory, we have conducted a variety of studies to identify some of the environmental risk factors. In several recent studies, we investigated the interactions between different environmental chemicals for induction of chromosome aberrations *in vivo*. We concentrated our effort on the interaction between a ubiquitous environmental carcinogen, benzene, and commonly used drugs and pharmaceutical chemicals (25). We reported that a widely used antischistosomal drug, praziquantel, can enhance the metabolism of benzene to form active metabolites and can enhance the induction of micronuclei in bone marrow cells of mice (26). A commonly used emulsifier in the pharmaceutical, cosmetic, and animal-raising industries, cremophore, can also enhance the clastogenic effect of benzene (27). We found that the enhancement effect of cremophore is correlated with a significant induction of cytochrome P-450I family of enzymes and the increased presence of activated benzene metabolites *in vivo*. On the other hand, co-administration of benzene with dimethylsulfoxide reduced the metabolism of benzene and its clastogenic activities (28). Because dimethylsulfoxide is a good free-radical scavenger, the latter observation indicates that metabolic activation of benzene may require the

formation of free radicals. Our studies and those of others clearly indicate the significant and complex contribution of environmental carcinogens in causation of cancer in humans.

Multiple Causes and Multiple Processes in Carcinogenesis

It is well known that not everyone who is exposed to similar concentrations of the same carcinogen will ultimately develop cancer. In fact, data from various epidemiological studies suggest that approximately 1 out of 10 heavy cigarette smokers eventually developed lung cancer. There is no doubt that some of the risk factors mentioned earlier contribute to cancer outcome. The fact that histologically identical cancer can be caused by different etiological agents and that cancer develops through a multistage process allows the documented risk factors to influence the outcome at different stages and, perhaps, with different mechanisms. Therefore, it is difficult to estimate cancer risk quantitatively even though the risk factors are identified. On the other hand, by documenting the biological/genetic events that are essential for carcinogenic process, it is possible to estimate cancer risk based on biomarker analysis. This approach is believed to be more precise than the estimate based on risk factors.

One of the well-documented genetic events in development of cancer in humans was discovered by Vogelstein and his colleagues (29). They found that alteration of several genes are required for the development of colorectal cancer. A typical sequence of events involves the alteration of chromosome 5 followed by mutagenic activation of *Ki-ras* oncogene and alteration of chromosomes 18 and 17.

Although the mechanisms involved in these specific genetic alterations are not yet elucidated, it has been hypothesized that certain genes may contain mutation hot spots so that they can be mutated readily after exposure to carcinogenic agents. The observed involvement of the mutated *p53* tumor-suppressor gene with different mutation spectra in different types of human cancer (30) is consistent with this hypothesis. On the other hand, it is difficult to provide an explanation for the mechanisms for multiple genetic alterations in the multistep carcinogenic process. Experimentally, it has been adequately demonstrated that an acute exposure to a carcinogen is sufficient to produce cancer in animals several months later. Based on this and other evidence, Loeb proposed that "an early step in tumor progression is one that induces a mutator phenotype" (31). He argued that the spontaneous mutation rate in somatic cells is not sufficient for the induction of multiple mutations in many cancers, but the induction of a mutator phenotype could increase the mutation rate and account for the phenomenon. It is reasonable to accept that the mutator phenotype is a general risk factor for development of cancer. A cell's inability to correctly repair DNA damage is one of the mechanisms that generate the mutator phenotype. In our laboratory, we have developed a challenge assay to detect the infidelity of DNA repair phenomenon. The details of this approach are presented in a later section of this paper.

Genetic Alterations in Mouse Mammary Tumors

Use of human tissues to document genetic events in carcinogenesis is limited by the availability of materials. In most cases, specimens can be obtained only from tumors that are at the end of the carcinogenic process. For this reason, many scientists turn to using experimental tumor models to document changes during the entire process. We have used an *in vivo-in vitro* mouse mammary tumor model for our study.

Our model for the study is based on the work of Ullrich and his colleagues (32,33). In this model, female mice were irradiated with a carcinogenic dose of 1 Gy of γ rays. At different times after the irradiation, but long before the development of mammary tumors, cells from the mammary glands were used to establish cell lines. The cells were characterized for their ability to populate mammary glands and for their tumorigenic phenotype. Many cell lines at various stages in the transformation process were established and stored in liquid nitrogen. Two lines (11A1 and 137V8C15) developed from nontumorigenic to tumorigenic phenotypes within approximately 25 *in vitro* passages.

The cell line 11A1 was derived from a mammary gland of an irradiated mouse 4 weeks after the irradiation. After the cells became established in culture, they were injected into the fat pad region of mammary glands in virgin (21 days old) female mice to test for their ability to produce normal ductal outgrowth *in vivo*. The cells' ability to produce ductal growth indicates that they were mammary cells. Cells with diploid DNA content were selected for further propagation *in vitro* and subcloned cell lines were derived from them. Cell line 11A1 is one of the subclones. We studied these cells using cytogenetic and molecular techniques to document changes that are related to the malignant transformation process.

A summary of our observations from analysis of the 11A1 cell line is shown in Table 1. At passage 6, cytogenetic analyses showed that these cells had a near tetraploid modal chromosome number. In addition, high frequencies of chromosome breakage and rearrangement were observed. At later passages, the chromosome numbers were reduced to near diploid. At passage 42, the chromosome number is 40; however, several rearranged chromosomes were detected by chromosome banding procedures. One large metacentric marker chromosome was formed.

Table 1. Genetic alterations in irradiated mouse mammary cells during the transformation process.^a

Observed changes	<i>In vitro</i> passage number ^b			
	6	11	24	42
Model chromosome number	78	74	41	40
% Metacentric translocation	50	37	90	100
<i>Rb</i> gene inactivation	—	+	++	++
<i>myc</i> Amplification	—	—	+	++
Tumorigenesis	—	—	+	+

^aCell line 11A1 was established from mammary glands of mice 4 weeks after 1 Gy of γ -irradiation.

^b(—) Negative response; (+) positive response; (++) more intense positive response.

The same passages of cells were evaluated using molecular techniques. Isolated DNA from various passages of cells were cut by *MspI* restriction enzyme, separated by agarose gel electrophoresis, and hybridized with ^{32}P -labeled *Rb* probes. The banding patterns of the Southern blot analyses show that the *Rb* gene is mutated in the early passages, from passage 11 to 42 [Table 1 (34,35)]. This led to inactivation of the gene. The latter is confirmed by Northern blot analysis for the lack of *Rb* messenger RNA and by immunofluorescent analysis for the lack of *Rb* protein (data not presented). Southern blot analyses for alterations of several oncogenes show that only the *c-myc* oncogene is amplified (Table 1). The amplification is correlated with the presence of double minute chromosomes in the same passage of cells. The amplification of the *c-myc* oncogene occurred in cells at a later passage than the inactivation of the *Rb* gene but at the same time when the cells became tumorigenic upon injection into host mice.

Our data indicate that the sequence of genetic events for mouse mammary cells to acquire tumorigenic potential is genetic instability, inactivation of the *Rb* tumor-suppressor gene, and amplification of the *c-myc* oncogene. Our observation provides further evidence for the involvement of several cellular genes in the carcinogenic process. Our observed genetic instability is consistent with the "mutator phenotype" as a crucial event in the process. It is very likely that the observed sequence of alterations is initiated by exposure of mammary cells to γ rays *in vivo* before cell lines were established. Affected cells have gene mutations and/or chromosome abnormalities, which interfere with normal cellular functions. One of them may be fidelity of DNA repair. We have developed a challenge assay to detect infidelity of DNA repair.

Cytogenetic Detection of Infidelity of DNA Repair

It is well accepted that inability to repair damaged DNA is a fundamental mechanism to produce cancer. This phenomenon is well documented in patients who have DNA repair deficiencies and are predisposed to develop cancer (e.g., patients with xeroderma pigmentosum and Bloom syndromes). Because cells have different efficiencies for repair of damage in different genes (36), general DNA repair deficiencies may produce mutation in specific genes. Therefore, infidelity of DNA repair may be a crucial mechanism in the generation of multiple, specific genetic alterations in the multistep carcinogenic process. However, a simple and efficient assay to detect infidelity of DNA repair is not available. We have developed a challenge assay by using cytogenetic techniques to address the DNA repair problems.

We hypothesize that physical and chemical agents that can react with DNA and proteins (e.g., DNA repair enzymes) can, even at very low doses, interfere with DNA repair processes. If these cells are subsequently challenged by exposing them to a defined dose of γ rays that can induce a finite amount of DNA lesions, the cells will have problems repairing the radiation-induced damage correctly. DNA fragments may not rejoin to the original

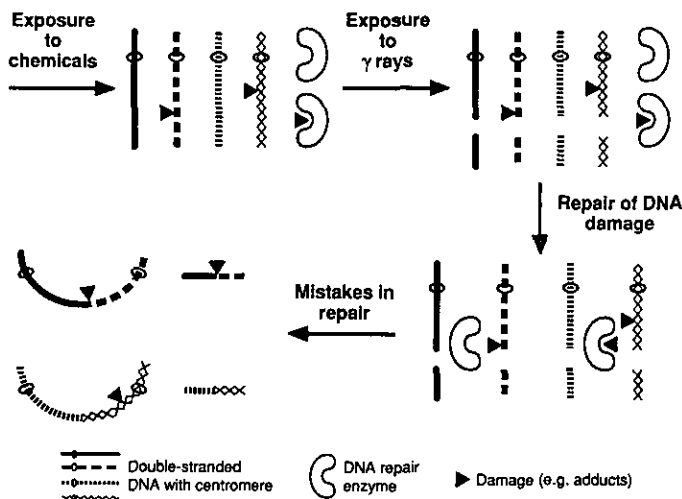


FIGURE 1. Hypothesized processes for infidelity of chromosome/DNA repair after exposure to carcinogens. The formation of rearranged DNA molecules that can be identified as dicentric chromosomes and acentric fragments is shown.

molecules. These abnormally repaired products can be detected as chromosome-type aberrations. Because chromosome-type abnormalities (e.g. dicentric chromosomes) are formed soon after irradiation and before DNA replication (37), we assume that the observed chromosome-type aberrations indicate infidelity of DNA repair. The basis for our hypothesis is diagrammatically illustrated in Figure 1. Furthermore, as described earlier, many cancer predisposition syndromes are due to DNA repair deficiencies. Therefore, cells from these patients will have increased chromosome rearrangements after their cells are exposed to agents such as ionizing radiation.

In one of our recent studies, lymphocytes from age- and sex-matched cigarette smokers and nonsmokers were investigated using our challenge assay (38). Several irradiation conditions were used. In one condition, cells were irradiated with a single dose of 100 cGy of X-rays during the G_0 phase of the cell cycle. Other cells were irradiated with two doses of 100 cGy each separated by 15 or 60 min. Unirradiated cells served as controls. We found that the chromosome translocation frequencies were consistently higher in smokers compared to nonsmokers under all three irradiation conditions ($p < 0.38$; $p < 0.10$; $p < 0.05$, respectively). Our data indicate that exposure to cigarette smoke can induce infidelity of DNA repair, and this abnormality may be one of the mechanisms for induction of serious health problems among smokers.

Mitomycin C (MMC) is known to form crosslinks with DNA and proteins, and we have used this chemical as a positive control to test our challenge assay. Lymphocytes from normal volunteers were exposed to 0, 0.01, and 0.1 $\mu\text{g/mL}$ of MMC. One hour later, cells were washed free of the chemical and irradiated 30 min later with γ rays. As shown in Figure 2, MMC caused significant enhancement of radiation-induced chromosome rearrangements. The

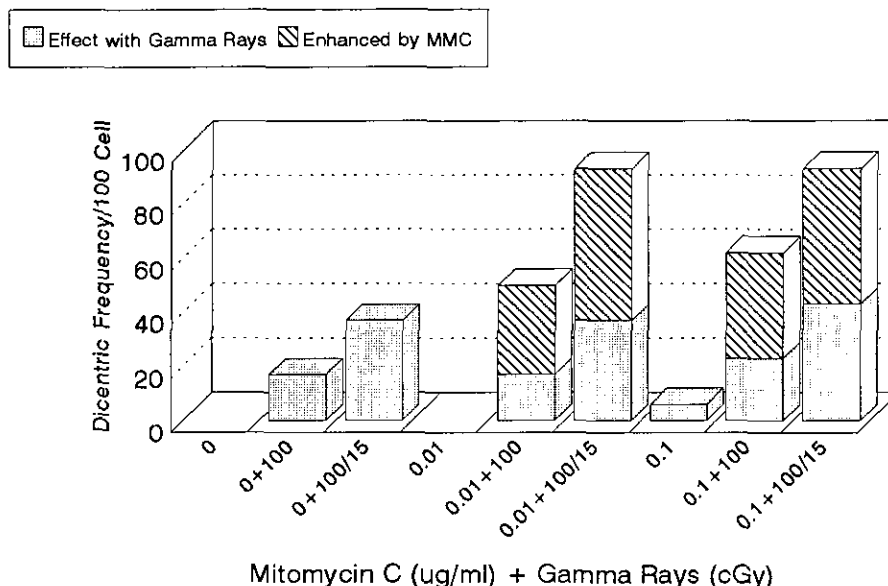


FIGURE 2. Induction of infidelity of DNA repair in human lymphocytes after exposure to mitomycin C (MMC). Cells were exposed to 0, 0.01, or 0.1 $\mu\text{g}/\text{mL}$ for 1 hr, washed, and irradiated 1 hr later with one dose of 100 cGy or two doses of 100 cGy (each separated by 15 min) of γ rays.

enhancement is detected with exposure to low doses of MMC, even when MMC does not induce chromosome aberrations by itself (0.01 $\mu\text{g}/\text{mL}$). Our data indicate, as expected, that crosslinks from exposure to MMC can cause infidelity of DNA repair.

It has been known for some time that some carcinogens are not genotoxic as defined by standard genotoxicity assays. Such carcinogens are generally known as non-genotoxic carcinogens (39). One nongenotoxic carcinogen is nickel. We have conducted a study with nickel acetate to determine whether this chemical can induce infidelity of DNA repair (40,41). Lymphocytes were exposed to 0.1–1000 μM nickel acetate for 1 hr, washed, and then irradiated 30 min later with two 75 cGy γ rays separated by 60 min. The result is summarized in Figure 3. Figure 3 shows a significant dose-dependent enhancement of radiation-induced chromosome rearrangements (analysis of variance: $p < 0.02$). A reduction of the enhancement effect at the high dose is probably due to toxicity of nickel to cells and/or to the repair processes.

Lymphocytes from patients who are predisposed to develop cancer are also used to investigate their efficiencies for DNA repair. These patients have Basal Cell Naevus Syndrome or epidermodysplasia verruciformis (EV). In addition to using γ rays, UV light was used as a challenging agent. Cells from both types of patients were exposed to γ rays in our challenge assay, but only cells from EV patients were exposed to UV light. Our data indicate that cells from both types of patients were not excessively deficient in repairing γ ray-induced DNA damage, whereas EV cells enhanced the UV-light-induced chromosome abnormalities by 4-fold over response from normal individuals (42). The extreme sensitivity of EV cells to UV light is consistent with their predisposition to UV-light induced skin cancer.

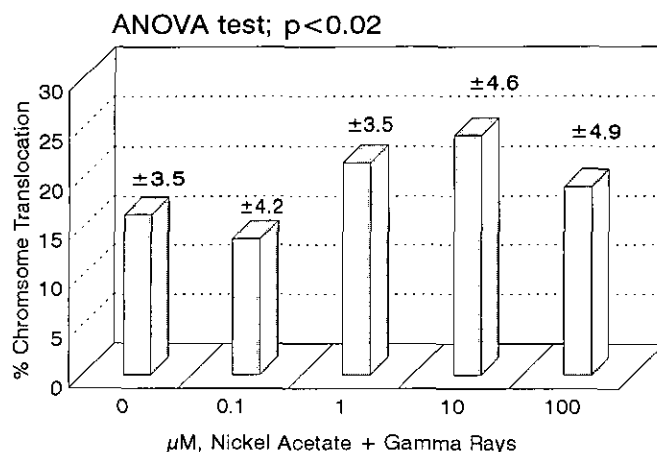


FIGURE 3. Induction of infidelity of DNA repair in human lymphocytes after exposure to nickel acetate. Cells were exposed to 0–100 μM nickel acetate for 1 hr, washed, and irradiated 1.5 hr later with two doses of 75 cGy (separated by 60 min) of γ rays.

Conclusion

Evidence was presented to emphasize the need to document factors that contribute to the development of cancer. Although it is possible to prioritize cancer risk using this and other data, additional information is needed for quantitative risk evaluation. Information is needed on the identification of biomarkers that predict carcinogenic outcome. Information is also needed on DNA repair problems and altered expression of cancer-related genes such as tumor-suppressor genes and oncogenes.

The development of a challenge assay to detect infidelity of DNA repair was presented, and some substantive

data were provided. It can be envisioned that infidelity of DNA repair can generate multiple genetic changes and therefore provide an opportunity for the alterations that are relevant to the development of cancer to evolve. Infidelity of DNA repair is potentially a mutator phenotype that is responsible for the generation of multiple genetic alterations in the multistep carcinogenic process. This and other relevant biomarkers may be useful for estimating cancer risk with additional precision.

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